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(21) International Application Number: PCT/US89/04616 (22) International Filing Date: 13 October 1989 (13.10.89) (30) Priority data: <table border="0"> <tr> <td>257,998</td> <td>14 October 1988 (14.10.88)</td> <td>US</td> </tr> <tr> <td>282,328</td> <td>9 December 1988 (09.12.88)</td> <td>US</td> </tr> <tr> <td>317,941</td> <td>2 March 1989 (02.03.89)</td> <td>US</td> </tr> <tr> <td>376,555</td> <td>7 July 1989 (07.07.89)</td> <td>US</td> </tr> <tr> <td>397,169</td> <td>21 August 1989 (21.08.89)</td> <td>US</td> </tr> </table> (71) Applicant: ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND [US/US]; 1430 Tulane Avenue, New Orleans, LA 70112 (US). (72) Inventors: COY, David, H. ; 4319 Perrier Street, New Orleans, LA 70115 (US). MOREAU, Jacques-Pierre ; 159 Westboro Street, Upton, MA 01568 (US). TAYLOR, John, E. ; 74 Fisk Hill Road, Upton, MA 01568 (US). KIM, Sun, Hyuk ; 20 Whitney Street, Chestnut Hill, MA 02167 (US).		257,998	14 October 1988 (14.10.88)	US	282,328	9 December 1988 (09.12.88)	US	317,941	2 March 1989 (02.03.89)	US	376,555	7 July 1989 (07.07.89)	US	397,169	21 August 1989 (21.08.89)	US	(74) Agent: KENNEDY, Bill; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111-2658 (US). (81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK, FI, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent). Published <i>With international search report.</i>
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(54) Title: THERAPEUTIC PEPTIDES (57) Abstract <p>In general, the invention features a linear (i.e., non-cyclic) bombesin analog of biologically active mammalian gastrin-releasing peptide (GRP) and amphibian bombesin, having an active site and a binding site responsible for the binding of the peptide to a receptor on a target cell; cleavage of a peptide bond in the active site of naturally occurring bombesin or GRP is unnecessary for <i>in vivo</i> biological activity. The analog has one of the following modifications: (a) a deletion of a residue within the active site and a modification of a residue outside of the active site, or (b) a replacement of one or two residues within the active site with a synthetic amino acid. The analog is capable of binding to the receptor and acting as a competitive inhibitor of the naturally occurring peptide by binding to the receptor and, by virtue of one of the modifications, failing to exhibit the <i>in vivo</i> biological activity of the naturally occurring peptide.</p>																	

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THERAPEUTIC PEPTIDES

Background of the Invention

5 This invention relates to therapeutic peptides useful, e.g., for treatment of benign or malignant proliferation of tissue and for gastrointestinal disorders.

The amphibian peptide bombesin, pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH₂ (Anastasi et al., *Experientia* 27:166-167 (1971)), is
10 closely related to the mammalian gastrin-releasing peptides (GRP), e.g., the porcine GRP, H₂N-Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-(NH₂) (McDonald et al., *Biochem.*
15 *Biophys. Res. Commun.* 90:227-233 (1979)) and human GRP, H₂N-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met (NH₂). Bombesin has been found to be a growth factor for a number of human cancer cell lines, including
20 small-cell lung carcinoma (SCLC), and has been detected in human breast and prostate cancer (Haveman et al., eds. Recent Results in Cancer Research - Peptide Hormones in Lung Cancer, Springer-Verlag, New York:1986). A number of these cancers are known to
25 secrete peptide hormones related to GRP or bombesin. Consequently, antagonists to bombesin have been proposed as agents for the treatment of these cancers.

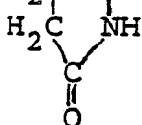
Cuttitta et al. demonstrated that a specific
monoclonal antibody to bombesin inhibited in vivo the
30 growth of a human small-cell lung cancer cell line xenografted to nude mice (Cuttitta et al., *Cancer Survey*

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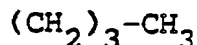
4:707-727 (1985)). In 3T3 murine fibroblasts which are responsive to the mitotic effect of bombesin, Zachary and Rozengurt observed that a substance P antagonist (Spantide) acted as a bombesin antagonist (Zachary et al., Proc. Natl. Acad. Sci. (USA), 82:7616-7620 (1985)). Heinz-Erian et al. replaced His at position 12 in bombesin with D-Phe and observed bombesin antagonist activity in dispersed acini from guinea pig pancreas (Heinz-Erian et al., Am. J. of Physiol. 252:G439-G442 (1987)). Rivier reported work directed toward restricting the conformational freedom of the bioactive C-terminal decapeptide of bombesin by incorporating intramolecular disulfide bridges; however, Rivier mentioned that, so far, bombesin analogs with this modification fail to exhibit any antagonist activity (Rivier et al., "Competitive Antagonists of Peptide Hormones," in Abstracts of the International Symposium on Bombesin-Like Peptides in Health and Disease, Rome, Italy (October, 1987)).

20 Abbreviations (uncommon):

pGlu = $\text{H}_2\text{C}--\text{CH}-\text{COOH}$ (pyroglutamic acid);



25 Nle = $\text{H}_2\text{N}-\text{CH}-\text{COOH}$ (norleucine)



Pal = 3-pyridyl-alanine

β -leu = β - homoleucine

γ -leu = gamma - homoleucine

30 D-Cpa = D-p-chlorophenylalanine

HyPro = hydroxyproline

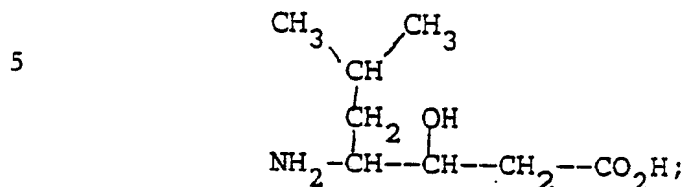
Nal = naphthylalanine

Sar = sarcosine

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Sta (statine) =

(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid,
and has the chemical structure:



AHPPA =

(3S, 4S)-4-amino-3-hydroxy-5-phenylpentanoic acid

ACHPA =

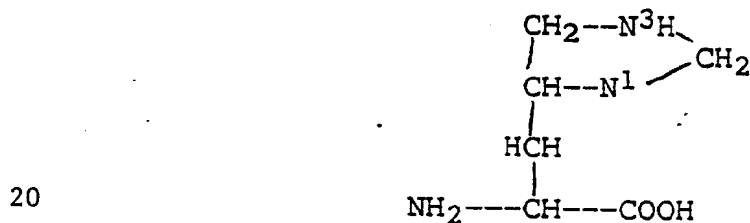
(3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid;

R = right (D) configuration; S = left (L) configuration;

racemate = equal mix of R and S

1-methyl-His; 3-methyl-His = methyl (CH₃) group on

nitrogen at positions 1 or 3 of Histidine:



Summary of the Invention

A linear peptide (i.e., noncyclic) which is an analog of naturally occurring, biologically active amphibian bombesin or mammalian gastrin releasing peptide (GRP) having an active site and a binding site responsible for the binding of the peptide to a receptor on a target cell, cleavage of a peptide bond in the active site of naturally occurring bombesin or GRP being unnecessary for in vivo biological activity, the analog having one of the following modifications: (a) a deletion of a residue within the active site and a modification of a residue outside of the active site, or (b) a replacement of one or two residues within the

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active site with a synthetic amino acid.

The analog is capable of acting as a competitive inhibitor of the naturally occurring peptide by binding to the receptor and, by virtue of one of the modifications, failing to exhibit the in vivo biological activity of the naturally occurring peptide.

The locations of the modifications that give rise to antagonists are determined by the location of the active site in the naturally occurring peptide. For example, the linear peptides for which introduction of a non-peptide bond between two residues, or the replacement of two natural amino acids with a synthetic β - or γ - amino acid, or the deletion ("des") of the C-terminal residue are useful in creating or enhancing antagonist activity are those in which activity is associated with the two C-terminal residues of the amino acid chain. Therefore, the active site of the naturally occurring peptide of which the peptides of the invention are analogs preferably includes at least one amino acid in the carboxy terminal half of the peptide, and the linear peptide of the invention includes that amino acid in its carboxy terminal half. Similarly, where the active site is located in the amino terminal portion of the naturally occurring peptide, the corresponding analogs of the invention will possess modifications in their amino terminal portions.

In preferred embodiments, the active site includes at least one amino acid residue located in the carboxyl terminal half of the naturally occurring biologically active peptide and that amino acid residue is located in the carboxyl terminal half of the linear peptide.

In other preferred embodiments, the active site includes at least one amino acid residue located in the

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amino terminal half of the naturally occurring biologically active peptide and that amino acid residue is located in the amino terminal half of the linear peptide.

5 Modifications can be introduced in a region involved in receptor binding, or in a non-binding region. Preferably, analogs of the invention are 25% homologous, most preferably, 50% homologous, with the naturally occurring peptides.

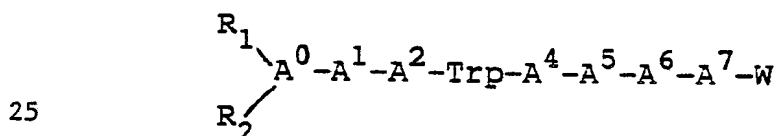
10 The analogs of the invention may have one of the modifications given in the generic formulae given below; either a non-peptide bond instead of a peptide bond between an amino acid of the active site and an adjacent amino acid; or a statine or AHPPA or ACHPA
15 residue, or a β - or γ - amino acid in place of one or two natural amino acids; or a deletion of the C-terminal amino acid which may or may not be accompanied by the addition of a substituent on the actual C-terminal group or the presence of an N-terminal residue that is not the
20 natural N-terminal amino acid of the peptides from which the analogs are derived. (Statine, AHPPA, and ACHPA have the chemical structures defined above. Where statine is used herein, AHPPA or ACHPA may also be used.)

25 By non-peptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e., $\text{CH}_2\text{-NH}$; or, less preferably, that the residue bonded to the carbon atom has a sulfur atom or a methylene carbon in place of its amino group, i.e.,
30 $\text{CH}_2\text{-S}$ or CO-CH_2 . (A detailed discussion of the chemistry of non-peptide bonds is given in Coy et al. (1988) Tetrahedron 44,3:835-841, hereby incorporated by reference.)

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One modification of the naturally occurring peptide to create an antagonist is of the amino terminal end of the molecule, which may be an electron-donating residue, e.g., a nitrogen-containing amino acid, such as those described for the amino terminal positions in the generic formulae below; for example, the N-terminal amino acid, which is A^0 or, if A^0 is deleted, is A^1 or, if A^0 and A^1 are deleted, is A^2 below, may be an aromatic D-isomer, or may be an alkylated amino acid. (Where "D" is not designated as the configuration of an amino acid, L is intended.) Another modification is of the C-terminal residue, which may be any of the "W" groups described below.

The invention includes a therapeutic peptide comprising between seven and nine amino acid residues, inclusive, the peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) neuromedin; (c) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (d) the ten amino acid carboxy-terminal region of amphibian bombesin, the therapeutic peptide being of the formula:

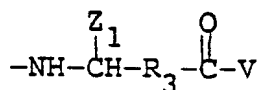


wherein

A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

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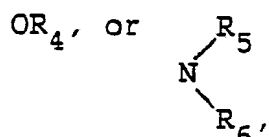
- A^1 = the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;
- A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;
- A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α -aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;
- A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β -Nal;
- A^6 = Sar, Gly, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;
- A^7 = 1-methyl-His, 3-methyl-His, or His;
- provided that if A^0 is present, A^1 cannot be pGlu; and if A^0 or A^1 is present, A^2 cannot be pGlu; and when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that W can be:



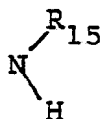
- wherein R_3 is $\text{CHR}_{14}-(\text{CH}_2)_{n1}$ (where R_{14} is either H or OH; and $n1$ may be either of 1 or 0), or is deleted, and Z_1 is the identifying group of any one of

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the amino acids Gly, Ala, cyclohexyl-Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F, Cl, Br, NO₂, OH, or CH₃), Trp, Cys, Met, Pro, HyPro, or isopropyl, cyclohexylmethyl, β-nal, β-naphthylmethyl, or phenylmethyl; and V is either



where R₄ is any of C₁₋₂₀ alkyl, C₃₋₂₀ alkenyl, C₃₋₂₀ alkynyl, phenyl, naphthyl, or C₇₋₁₀ phenylalkyl, and each R₅, and R₆, independently, is any of H, C₁₋₁₂ alkyl, C₇₋₁₀ phenylalkyl, lower acyl, or



where R₁₅ is any of H, C₁₋₁₂ alkyl, C₇₋₁₀ phenylalkyl, or lower acyl; provided that when one of R₅ or R₆ is NHR₁₅, the other is H; and provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R₁ and R₂, independently, is H, C₁₋₁₂ alkyl, C₇₋₁₀ phenylalkyl, COE₁ (where E₁ is C₁₋₂₀ alkyl, C₃₋₂₀ alkenyl, C₃₋₂₀ alkynyl, phenyl, naphthyl, or C₇₋₁₀ phenylalkyl), or lower acyl, and R₁ and R₂ are bonded to the N-terminal amino acid of the peptide, and further provided that when one of R₁ or R₂ is COE₁, the other must be H, or a pharmaceutically acceptable salt thereof.

Preferably, the therapeutic peptide has the formula wherein

A⁰ = Gly, D-Phe, or is deleted;
 A¹ = p-Glu, D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;
 A² = Gln, His, 1-methyl-His, or 3-methyl-His;
 A⁴ = Ala;

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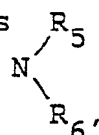
$A^5 = \text{Val};$

$A^6 = \text{Sar, Gly, D-Phe, or D-Ala};$

$A^7 = \text{His};$

provided that where R_3 is $\text{CH}_2\text{-CH}_2$, Z_1 is the
 5 identifying group of Leu or Phe; or where R_3 is CH_2 ,
 Z_1 is the identifying group of β -Leu or Leu; or where
 R_3 is CHOH-CH_2 , Z_1 is the identifying group of Leu
 or is isopropyl, cyclohexylmethyl, β -naphthylmethyl, or
 phenylmethyl; provided that V is

10



and each R_5 and R_6 is H.

Preferably, the peptide is of the generic
 formula wherein V is NHR_6 where R_6 is NH_2 .

15

Most preferably the therapeutic peptide has the
 following amino acid formulas:

pGlu-Gln-Trp-Ala-Val-Gly-His-statine-amide; and
 D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His- β -Leu- NH_2 .

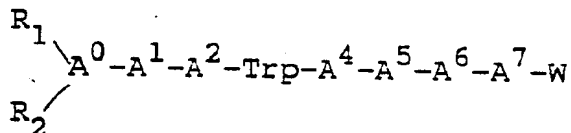
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The invention also includes a therapeutic
 peptide comprising between eight and ten amino acid
 residues, inclusive, the peptide being an analog of one
 of the following naturally occurring peptides
 terminating at the carboxy-terminus with a Met residue:

25

(a) litorin; (b) neuromedin; (c) the ten amino acid
 carboxy-terminal region of mammalian gastrin releasing
 peptide; and (d) the ten amino acid carboxy-terminal
 region of amphibian bombesin, the therapeutic peptide
 being of the formula:

30



wherein

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A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

A^1 = the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α -aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β -Nal;

A^6 = Sar, Gly, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

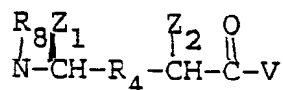
provided that if A^0 is present, A^1 cannot be pGlu;

and if A^0 or A^1 is present, A^2 cannot be pGlu; and

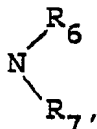
when A^0 is deleted and A^1 is pGlu, R_1 must be H

and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that W can be:

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wherein R_4 is CH_2-NH , CH_2-S , $CO-CH_2$, or CH_2-CH_2 , and each of Z_1 and Z_2 , independently, is the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, β -Nal, p-X-Phe (where $X = H, F, Cl, Br, NO_2, OH$ or CH_3), Trp, Cys, Met, Pro, HyPro, cyclohexyl-Ala, or cyclohexylmethyl; provided that where R_4 is CH_2-NH and Z_2 is the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where $X = H, F, Cl, Br, NO_2, OH$ or CH_3), Trp, Cys, Met, Pro, HyPro, or cyclohexylmethyl, Z_1 can only be the identifying group of any one of the amino acids Ser, Asp, Glu, Cys, Pro, HyPro, or cyclohexylmethyl; and provided that where R_4 is CH_2-NH and Z_1 is the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where $X = H, F, Cl, Br, NO_2, OH$ or CH_3), Trp, Cys, Met, Pro, or HyPro, Z_2 can only be the identifying group of any one of the amino acids Ser, Asp, Glu, Cys, Pro, HyPro, or cyclohexylmethyl; and V is either OR_5 or



where each R_8 , R_5 , R_6 , and R_7 , independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; and provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2 , independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1

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is C₁₋₂₀ alkyl, C₃₋₂₀ alkenyl, C₃₋₂₀ alkynyl, phenyl, naphthyl, or C₇₋₁₀ phenylalkyl), or lower acyl, and R₁ and R₂ are bonded to the N-terminal amino acid of the peptide, and further provided that
 5 when one of R₁ or R₂ is COE₁, the other must be H, or a pharmaceutically acceptable salt thereof.

Preferably, the therapeutic peptide has the formula wherein

A⁰ = Gly, D-Phe, or is deleted;
 10 A¹ = p-Glu, D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;
 A² = Gln, His, 1-methyl-His, or 3-methyl-His;
 A⁴ = Ala;
 A⁵ = Val;
 A⁶ = Sar, Gly, D-Phe, or D-Ala;
 15 A⁷ = His;

where R₄ is CH₂-NH, each Z₁ is cyclohexylmethyl or is the identifying group of Leu or Phe; or Z₂ is the identifying group of Met, Leu or Phe.

Most preferably, the therapeutic peptide
 20 includes D-β-Nal at position A¹, where each of Z₁ and Z₂, independently, is Leu or Phe.

Examples of such peptides are:

D-β-Nal-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH₂NH]Leu-NH₂,
 or
 25 D-β-Nal-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH₂NH]Phe-NH₂.

In addition, the therapeutic peptide may include where R₄ is CH₂-NH, and the carbon atom bonded to Z₂ is of the R configuration. An example of such a peptide is

30 D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH₂NH]-D-Phe-NH₂.

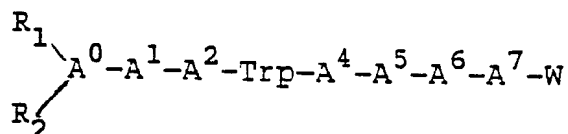
In addition, the peptide may include where A⁰ or A¹ is a D amino acid, V is OR₄; for example, a methylester derivative such as the therapeutic peptide D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Met-methylester.

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The invention also includes a therapeutic peptide comprising between seven and nine amino acid residues, inclusive, the peptide being an analog of one of the following naturally occurring peptides

5 terminating at the carboxy-terminus with a Met residue:
 (a) litorin; (b) neuromedin; (c) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (d) the ten amino acid carboxy-terminal region of amphibian bombesin, the therapeutic peptide

10 being of the formula:



wherein

- 15 $A^0 =$ Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;
- 20 $A^1 =$ the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;
- 25 $A^2 =$ pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;
- $A^4 =$ Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle,
- 30 α -aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;

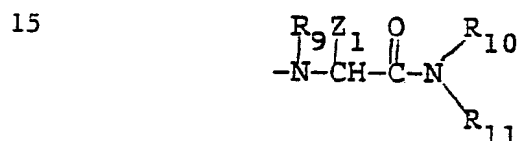
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$A^5 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, p-X-Phe (where
 X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or
 β -Nal;

5 $A^6 =$ Sar, Gly, or the D-isomer of any of Ala, Val,
 Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F,
 Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or
 β -Nal;

$A^7 =$ 1-methyl-His, 3-methyl-His, or His;

10 provided that if A^0 is present, A^1 cannot be pGlu;
 and if A^0 or A^1 is present, A^2 cannot be pGlu; and
 when A^0 is deleted and A^1 is pGlu, R_1 must be H
 and R_2 must be the portion of Glu that forms the imine
 ring in pGlu; and further provided that W can be:



wherein Z_1 is the identifying group of any one of the
 amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu,
 20 Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2 , OH or
 CH_3), Trp, Cys, Met, Pro, or HyPro; and each R_9 ,
 R_{10} , and R_{11} , independently, is H, lower alkyl,
 lower phenylalkyl, or lower naphthylalkyl; and provided
 that any asymmetric carbon atom can be R, S or a racemic
 25 mixture; and further provided that each R_1 and R_2 ,
 independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl,
 COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl,
 C_{3-20} alkynyl, phenyl, naphthyl, or C_{7-10}
 phenylalkyl), or lower acyl, and R_1 and R_2 are
 30 bonded to the N-terminal amino acid of the peptide, and
 further provided that when one of R_1 or R_2 is
 COE_1 , the other must be H, or a pharmaceutically
 acceptable salt thereof.

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Preferably, the peptide includes wherein

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;

5 A^4 = Ala;

A^5 = Val;

A^6 = Sar, Gly, D-Phe, or D-Ala;

A^7 = His;

provided that Z_1 is the identifying group of any one
10 of the amino acids Leu or D or L p-X-Phe (where X = H,
F, Cl, Br, NO_2 , OH or CH_3); and each R_9 , R_{10} and
 R_{11} , independently, is H, lower alkyl, lower
phenylalkyl, or lower naphthylalkyl.

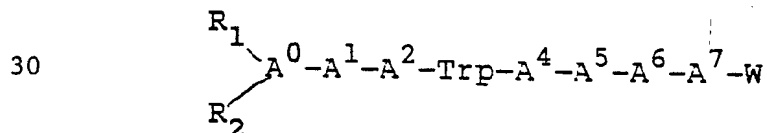
Most preferably, the peptide includes wherein

15 Z_1 is Leu, R_9 is H, and each R_{10} and R_{11} is
lower alkyl. Examples of such a peptide are:

D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ethylamide; or

D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu- NH_2 .

The invention also includes a therapeutic
20 peptide comprising between six and eight amino acid
residues, inclusive, the peptide being an analog of one
of the following naturally occurring peptides
terminating at the carboxy-terminus with a Met residue:
(a) litorin; (b) neuromedin; (c) the ten amino acid
25 carboxy-terminal region of mammalian gastrin releasing
peptide; and (d) the ten amino acid carboxy-terminal
region of amphibian bombesin, the therapeutic peptide
being of the formula:

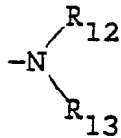


wherein

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- A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;
- A^1 = the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;
- A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;
- A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α -aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;
- A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β -Nal;
- A^6 = Sar, Gly, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;
- A^7 = 1-methyl-His, 3-methyl-His, or His;
- provided that if A^0 is present, A^1 cannot be pGlu; and if A^0 or A^1 is present, A^2 cannot be pGlu; and when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that W can be:

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wherein each R_{12} and R_{13} , independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2 , independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkynyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of the peptide, and further provided that when one of R_1 or R_2 is COE_1 , the other must be H, or a pharmaceutically acceptable salt thereof.

Preferably, the therapeutic peptide includes wherein

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, D-Ala, D- β -Nal, D-Cpa, or D-Asn;

A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

A^6 = Sar, Gly, D-Phe, or D-Ala;

A^7 = His;

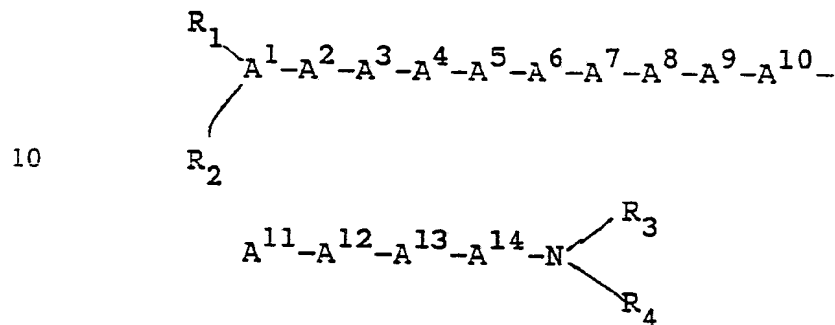
where each R_{12} and R_{13} , is H; and each R_1 and R_2 , independently, is H, lower alkyl, or lower acyl.

Preferably, where either of N_{12} or N_{13} is other than H, A^7 is His, A^6 is Gly, A^5 is Val, A^4 is Ala, and A^2 is His; and where either of R_1 or R_2 is other than H, A^1 must not be deleted.

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The invention also includes a bombesin therapeutic peptide of the formula:
pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Statine.

The invention also includes an effective bombesin antagonistic peptide containing the amino acid formula:



wherein

A¹ = pGlu, D or L, or is deleted;

15 $A^2 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 (X = F, Cl, Br, OH or CH_3), Trp,
 β -naphthylalanine or is deleted;

A³ = Arg, D-Arg, Lys, D-Lys or is deleted;

20 $A^4 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 (X = F, Cl, Br, OH or CH_3), Trp,
 β -naphthylalanine or is deleted ;

25 A⁵ = Gln, Asn, Gly, Ala, Leu, Ile, Nle,
α-aminobutyric acid, Met, Val, Phe, D-Phe,
p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp,
β-naphthylalanine, D-Ala or is deleted;

30 A⁶ = Gln, Asn, Gly, Ala, D-Ala, N-Ac-D-Ala, Leu,
Ile, Nle, α -aminobutyric acid, Met, Val, Phe,
p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp,
p-Glu, β -naphthylalanine or is deleted;

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- $A^7 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, D-Phe,
p-X-Phe (X = F, Cl, Br, OH or CH_3), Trp, Lys,
His, or β -naphthylalanine;
5 $A^8 =$ Trp or Met;
 $A^9 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
(X = F, Cl, Br, OH or CH_3), Trp, or
 β -naphthylalanine, D or L;
10 $A^{10} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
(X = F, Cl, Br, OH or CH_3), Trp, Thr, or
 β -naphthylalanine;
 $A^{11} =$ Gly, Phe, D or L;
15 $A^{12} =$ His, Phe, or p-X-Phe (X = F, Cl, Br, OH,
 CH_3), D or L;
 $A^{13} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
(X = F, Cl, Br, OH or CH_3), Trp, or
20 β -naphthylalanine;
 $A^{14} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
(X = F, Cl, Br, OH or CH_3), Trp, or
 β -naphthylalanine;

25 provided that

each R_1 , R_2 , R_3 , and R_4 , independently,
is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where
 E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkynyl,
phenyl, naphthyl, or C_{7-10} phenylalkyl), or $COOE_2$
30 (where E_2 is C_{1-10} alkyl or C_{7-10} phenylalkyl),
and R_1 and R_2 are bonded to the N-terminal amino
acid of the peptide, which can be A^1 , A^2 , A^3 ,
 A^4 , A^5 , A^6 , or A^7 , and further provided that
when one of R_1 or R_2 is COE_1 or $COOE_2$, the other

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must be H, and when one of R_3 or R_4 is COE_1 or COOE_2 , the other must be H, and further provided that when $A^1 = \text{pGlu}$, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and
 5 for each of the residues A^7 , A^8 , A^9 , A^{11} , A^{12} , and A^{13} , independently, the carbon atom participating in the amide bond between that residue and the nitrogen atom of the alpha amino group of the adjacent amino acid residue may be a carbonyl carbon or may be reduced to a
 10 methylene carbon, provided that at least one such carbon atom must be reduced to a methylene carbon,

where the peptide further comprises

$A^5 = \text{Cys}$;
 $A^6 = \text{Cys}$ or a D-isomer of any of the amino acids
 15 described for this position;
 $A^7 = \text{pGlu}$, Cys , 1-methyl-His, or 3-methyl-His;
 $A^9 = \text{Cys}$;
 $A^{11} = \text{Sar}$, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where $X = \text{F}$, Cl , Br , NO_2 ,
 20 OH, or CH_3), Trp, Cys, or β -Nal;
 $A^{12} = \text{1-methyl-His}$, or 3-methyl-His;
 and where A^{14} may be deleted.

Most preferably, this therapeutic peptide is of the formula:

25 D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ $[\text{CH}_2\text{NH}]$ Phe-NH₂

Preferably, the amino acid sequence of the therapeutic peptides of the formulas described herein are at least 25% homologous with the amino acid sequence of the naturally occurring peptide; most preferably,
 30 this homology is at least 50%.

(Non-peptide bonds in which the peptide bond is reduced are symbolized herein by " $\psi[\text{CH}_2\text{NH}]$ " or " ψ ".)

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Antagonists of the invention are useful for treating diseases involving the malignant or benign proliferation of tissue, such as all forms of cancer where bombesin-related or GRP-related substances act as autocrine or paracrine mitotic factors, e.g., cancers of the gastrointestinal tract, pancreatic cancer, colon cancer, lung cancer, particularly the small cell subtype, or breast cancer; or for treating arteriosclerosis, and disorders of gastrointestinal tissues related to gastric and pancreatic secretions and motility; for example, for causing the suppression of amylase secretion, or for appetite control.

In the generic formulae given above, when any one of R_1 - R_{13} or R_{15} is an aromatic, lipophilic group, the in vivo activity can be long lasting, and delivery of the compounds of the invention to the target tissue can be facilitated.

The identifying group of an α -amino acid is the atom or group of atoms, other than the α -carbonyl carbon atom, the α -amino nitrogen atom, or the H atom, bound to the asymmetric α -carbon atom. To illustrate by examples, the identifying group of alanine is CH_3 , the identifying group of valine is $(\text{CH}_3)_2\text{CH}$, the identifying group of lysine is $\text{H}_3\text{N}^+(\text{CH}_2)_4$ and the identifying group of phenylalanine is $(\text{C}_6\text{H}_5)\text{CH}_2$. The identifying group of a β - or γ -amino acid is the analogous atom or group of atoms bound to respectively, the β - or the γ -carbon atom. Where the identifying group of an amino acid is not specified it may be α , β , or γ .

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

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Description of the Preferred Embodiments

We first briefly describe the drawings.

Drawings

5 Fig. 1 is a graph of tumor growth curves for NCI-H69 xenografts.

Fig. 2 is a series of amino acid sequences of naturally occurring peptides of which peptides of the invention are analogs.

10 Fig. 3 is a graph showing the effect of injection of the bombesin analog D-Phe⁶BN(6-13)methylester on bombesin-stimulated pancreatic amylase assay.

We now describe the structure, synthesis, and use of the preferred embodiments of the invention.

15 Structure

The peptides of the invention all have a non-peptide bond in at least one of the indicated positions, except for the statine substituted analogs and β -leu, such as sta⁸-desLeu⁸-Met⁹ litorin. By
20 non-peptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon. The peptide bond reduction method which yields this non-peptide bond is described in Coy et al., U.S. patent
25 application, Serial No. 879,348, assigned to the same assignee as the present application, hereby incorporated by reference. Any one of the amino acids in positions 0, 1, 2, and 9 of the litorin antagonists may be deleted from the peptides, and the peptides are still active as
30 antagonists.

The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic,

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lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis of Litorin Analogs

The synthesis of the litorin antagonist pGlu-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH₂NH]Leu-NH₂ follows. Other antagonists of bombesin, litorin, neuromedin, or GRP can be prepared by making appropriate modifications of the following synthetic method.

The first step is the preparation of the intermediate

pGlu-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbonyl)-Leu ψ [CH₂NH]Leu-benzhydrylamine resin, as follows.

Benzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (0.97 g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid (TFA) in methylene chloride (2 times for 1 and 25 min. each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; and (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with alpha-t-butoxycarbonyl(Boc)-leucine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hour, and the resulting amino acid resin is then cycled through steps (a) to (f) in the above wash program. Boc-leucine aldehyde (1.25 mmoles), prepared by the method of Fehrentz and Castro, Synthesis, p. 676 (1983), is dissolved in 5 ml of dry dimethylformamide (DMF) and added to the resin TFA salt

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suspension followed by the addition of 100 mg (2 mmole) of sodium cyanoborohydride (Sasaki and Coy, Peptides 8:119-121 (1987); Coy et al., id.). After stirring for 1 hour, the resin mixture is found to be negative to
5 ninhydrin reaction (1 min.), indicating complete derivatization of the free amino group.

The following amino acids (1.5 mmole) are then coupled successively in the presence diisopropylcarbodiimide (1.5 mmole), and the resulting
10 amino acid resin is cycled through washing/deblocking steps (a) to (f) in the same procedure as above: Boc-His(benzyloxycarbonyl), Boc-Gly, Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled as a 6 M excess of the p-nitrophenylester), and pGlu. The completed resin is
15 then washed with methanol and air dried.

The resin described above (1.6 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream
20 of dry nitrogen, and free peptide is precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 mm) of Sephadex G-25 (Pharmacia Fine Chemicals, Inc.). Fractions containing a major
25 component by uv absorption and thin layer chromatography (TLC) are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of octadecylsilane-silica (Whatman LRP-1, 15-20 µm mesh size).

30 The peptide is eluted with a linear gradient of 0-30% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by TLC and analytical high performance liquid chromatography (HPLC) and pooled to give maximum purity. Repeated lyophilization of the

- 25 -

solution from water gives 60 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by HPLC and TLC. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide. The presence of the $\text{Leu}\psi[\text{CH}_2\text{-NH}]\text{Leu}$ bond is demonstrated by fast atom bombardment mass spectrometry.

pGlu-Gln-Trp-Ala-Val-Gly-His-Phe $\psi[\text{CH}_2\text{NH}]\text{Leu-NH}_2$ and

pGlu-Gln-Trp-Ala-Val-Gly-His-Leu $\psi[\text{CH}_2\text{NH}]\text{Leu-NH}_2$ or other peptides are prepared in similar yields in an analogous fashion by appropriately modifying the above procedure.

Solid phase synthesis of D-Phe¹, Leu⁸ $\psi[\text{CH}_2\text{NH}]\text{D-Phe}^9$ -litorin, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu $\psi[\text{CH}_2\text{NH}]\text{-D-Phe-NH}_2$, was carried out as follows:

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His(tosyl)-Leu $\psi[\text{CH}_2\text{NH}]\text{-D-Phe-benzhydrylamine resin}$ was synthesized first.

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc.) (1.25 g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of an Advanced ChemTech ACT 200 peptide synthesizer programmed to perform the reaction cycle described as steps (a) through (f) above.

The neutralized resin is stirred with Boc-D-phenylalanine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 h and the resulting amino acid resin is then cycled through steps (a) to (g) in the above wash program. The Boc group is then removed by TFA treatment. Boc-leucine aldehyde (1.25 mmoles), prepared by the method of Fehrentz and Castro (1), is dissolved in 5 ml of dry DMF and added to the resin TFA salt suspension followed by the addition of 100 mg (2

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mmoles) of sodium cyanoborohydride (2,3). After stirring for 1 h, the resin mixture is found to be negative to ninhydrin reaction (1 min) indicating complete derivatization of the free amino group.

5 The following amino acids (1.5 mmole) are then coupled successively by the same procedure:
Boc-His(benzyloxycarbonyl), Boc-Gly, Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled in the presence of 1 equiv. hydroxybenzotriazole), Boc-D-Phe (coupled in the presence
10 of 1 equiv. hydroxybenzotriazole). After drying, the peptide resin weighed 1.93 g.

 The resin (1.93 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is
15 evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 mm) of Sephadex G-25. Fractions containing a major component by uv
20 absorption and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane (10-15 μ M). This is eluted with a linear gradient of 15-45% acetonitrile in 0.1% trifluoroacetic acid in
25 water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 120 mg of the product as a white, fluffy powder.

30 Other peptides, e.g., D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH₂NH]-D-Phe-NH₂, may be synthesized using essentially the same procedure.

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Synthesis of Leu⁸ ψ [CH₂NH] D-Phe⁹ Litorin

Solid phase synthesis of

D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH₂NH]-D-Phe-NH₂
was carried out as follows:

5 Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His(tosyl)-Leu ψ [CH₂NH]-
D-Phe-benzhydrylamine resin was synthesized first.

Benzhydrylamine-polystyrene resin (Advanced
ChemTech, Inc.) (1.25 g, 0.5 mmole) in the chloride ion
form is placed in the reaction vessel of an Advanced
10 ChemTech ACT 200 peptide synthesizer programmed to
perform the following reaction cycle: (a) methylene
chloride; (b) 33% trifluoroacetic acid in methylene
chloride (2 times for 1 and 25 min each); (c) methylene
chloride; (d) ethanol; (e) methylene chloride; (f) 10%
15 triethylamine in chloroform.

The neutralized resin is stirred with
Boc-D-phenylalanine and diisopropylcarbodiimide (1.5
mmole each) in methylene chloride for 1 h and the
resulting amino acid resin is then cycled through steps
20 (a) to (g) in the above wash program. The Boc group is
then removed by TFA treatment. Boc-leucine aldehyde
(1.25 mmoles), prepared by the method of Fehrentz and
Castro (1), is dissolved in 5 ml of dry DMF and added to
the resin TFA salt suspension followed by the addition
25 of 100 mg (2 mmoles) of sodium cyanoborohydride (2,3).
After stirring for 1 h, the resin mixture is found to be
negative to ninhydrin reaction (1 min) indicating
complete derivatization of the free amino group.

The following amino acids (1.5 mmole) are then
30 coupled successively by the same procedure:
Boc-His(benzyloxycarbonyl), Boc-Gly, Boc-Val, Boc-Ala,
Boc-Trp, Boc-Gln (coupled in the presence of 1 equiv.
hydroxybenzotriazole), Boc-D-Phe (coupled in the
presence of 1 equiv. hydroxybenzotriazole). After
drying, the peptide resin weighed 1.93 g.

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The resin (1.93 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 mm) of Sephadex G-25. Fractions containing a major component by uv absorption and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane (10-15 µM). This is eluted with a linear gradient of 15-45% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 120 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide. The presence of the Leuψ[CH₂NH] peptide bond is demonstrated by fast atom bombardment mass spectrometry.

25 Synthesis of D-Phe¹-Des-Met⁹ Litorin

Solid phase synthesis of D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH₂ was carried out as follows.

Step (1): Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc. (0.62 gm, 0.25 mmole) in the chloride ion form is placed in the reaction vessel of an ACT 200 peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times

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for 1 and 25 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with
5 Boc-leucine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hr and the resulting amino acid resin is then cycled through steps (a) to (g) in the above wash program. The following amino acids (1.5 mmole) are then coupled successively by the same
10 procedure: Boc-His (benzyloxycarbonyl, Boc-Gly, Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled as a 6M excess of the p-nitrophenylester, and pGlu (coupled in the presence of hydroxybenzotriazole). After drying, the peptide resin weighed 0.92 g.

15 Step (2): The resin (0.92 g) is then mixed with anisole (5 ml), dithiothreitol (200 mg) and anhydrous hydrogen fluoride (35 ml) at 0° C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide
20 precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 cm) of Sephadex G-25. Fractions containing a major component by UV absorption and thin layer chromatography are then pooled,
25 evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane (10-15 microm). The column is eluted with a linear gradient of 0-30% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and
30 pooled to give maximum purity. Repeated lyophilization of the solution from water gives a white, fluffy powder; this product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide.

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Synthesis of

D-Nal-Gln-Trp-Ala-Val-Gly-His-Leu-NH₂ was accomplished using the same procedure as described above (0.62 g, 0.25 mmole of benzyhydramine resin in step (1), and 0.92 g in step (2)).

Synthesis of

N-acetyl-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH₂ was accomplished using the same procedure as that described above, using 0.62 g (0.25 mmole) of benzhydramine resin in step (1), and mixing 0.92 g of the resin with anisole in step(2), except that the final Boc group was removed and the resin acetylated with acetic anhydride in methylene chloride.

Synthesis of Sta⁸-Des-Met⁹ Litorin

A statine, AHPPA, or ACHPA residue can be substituted in place of any two amino acids of the peptide, where the peptide contains only peptide bonds. For example, sta⁸-des Met⁹ litorin was prepared in an analagous fashion by first coupling statine to the resin and then proceeding with the addition of Boc-His(benzylocarbonyl). Statine or Boc-statine can be synthesized according to the method of Rich et al., 1978, J. Organic Chem. 43; 3624; and Rich et al., 1980, J. Med. Chem. 23: 27, and AHPPA and ACHPA can be synthesized according to the method of Hui et al., 1987, J. Med. Chem. 30: 1287; Schuda et al., 1988, J. Org. Chem. 53:873; and Rich et al., 1988, J. Org. Chem. 53:869.

Solid-phase synthesis of the peptide BIM-26120, pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH₂, was accomplished through the use of the following procedures in which alpha-t-butoxycarbonyl statine (prepared by the procedure of Rich et al., J. Org. Chem. 1978, 43, 3624) is first coupled to methylbenzhydramine-polystyrene

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resin. After acetylation, the intermediate p-Glu-Gln-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbonyl)-Stat-methylbenzhydrylamine resin is prepared. The synthetic procedure used for this preparation follows in detail:

5 1. Incorporation of alpha-t-butoxycarbonyl statine on methylbenzhydrylamine resin.

Methylbenzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (1.0 g, 0.73 mmol) in the chloride ion form is placed in the reaction vessel of a Vega 250C
10 Coupler peptide synthesizer. The synthesizer was programmed to perform the following reactions: (a) methylene chloride; (b) 10% triethylamine in chloroform; (c) methylene chloride; and (d) dimethylformamide.

The neutralized resin is mixed for 18 hours
15 with the preformed active ester made from alpha-t-butoxycarbonyl statine (1.46 mmol), diisopropyl carbodiimide (2 mmol), and hydroxybenzotriazole hydrate (1.46 mmol in dimethylformamide at 0° C. for one hour. The resulting amino acid resin is washed on the
20 synthesizer with dimethylformamide and then methylene chloride. The resin mixture at this point was found by the Kaiser ninhydrin test (5 minutes) to have an 84% level of statine incorporation on the resin.

Acetylation was performed by mixing the
25 amino-acid resin for 15 minutes with N-acetyl imidazole (5 mmol) in methylene chloride. Derivatization to the 94-99% level of the free amino groups of the resin was indicated by the Kaiser ninhydrin test (5 minutes). The Boc-statine-resin is then washed with methylene chloride.

30 2. Couplings of the Remaining Amino Acids.

The peptide synthesizer is programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid (TFA) in methylene chloride (2 times for 5 and 25 min. each); (c)

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methylene chloride; (d) isopropyl alcohol; (e) 10% triethylamine in chloroform; and (f) methylene chloride.

The following amino acids (2.19 mmol) are then coupled successively by diisopropyl carbodiimide (4 mmol) alone or diisopropyl carbodiimide (4 mmol) plus hydroxybenzotriazole hydrate (1.47 or 0.73 mmol) and the resulting peptide-resin is washed on the synthesizer with dimethylformamide and then methylene chloride, and then cycled through the washing and deblocking steps (a) to (f) in the procedure described above.

Boc-His (benzyloxycarbonyl) (coupled in the presence of 2 equivalents hydroxybenzotriazole); Boc-Gly; Boc-Val; Boc-Ala and Boc-Trp (coupled as the preformed hydroxybenzotriazole active esters made by reaction at 0° C for one hour with 1 equivalent hydroxybenzotriazole hydrate); Boc-Gln and pGlu (also coupled as the preformed active esters of hydroxybenzotriazole made by reaction at 0° C for one hour with 1 equivalent hydroxybenzotriazole hydrate). The completed peptide-resin is then washed with methanol and air dried.

The peptide-resin described above (1.60 g, 0.73 mmol) is mixed with anisole (2.5 mL), dithioerythritol (50 mg), and anhydrous hydrogen fluoride (30 mL) at 0° C. for one hour. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, and the free peptide is precipitated and washed with ether. The crude peptide is dissolved in 100 mL of 1 M acetic acid and the solution is then evaporated under reduced pressure. The crude peptide is dissolved in a minimum volume of methanol/water 1/1 and triturated with 10 volumes of ethyl acetate.

The triturated peptide is applied to a column (9.4 mm I.D. x 50 cm) of octadecylsilane-silica (Whatman

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Partisil 10 ODS-2 M 9). The peptide is eluted with a linear gradient of 20-80% of 20/80 0.1% trifluoroacetic acid/acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by TLC and analytical high performance liquid chromatography (HPLC) and pooled to give maximum purity. Lyophilization of the solution from water gives 77 mg of the product as a white fluffy powder.

Other compounds including D-Cpa¹, β -leu⁸, desMet⁹ Litorin can be prepared as above and tested for effectiveness as agonists or antagonists in the following test program.

Phase 1 - 3T3 Peptide Stimulated [³H] Thymidine Uptake Assay

Cell Culture. Stock cultures of Swiss 3T3 cells are grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum in humidified atmosphere of 10% CO₂/90% air at 37°C. For experimental use, the cells are seeded into 24-well cluster trays and used four days after the last change of medium. The cells are arrested in the G1/G0 phase of the cell cycle by changing to serum-free DMEM 24 hours prior to the thymidine uptake assay.

Assay of DNA Synthesis. The cells are washed twice with 1ml aliquots of DMEM (-serum) then incubated with DMEM (-serum), 0.5 μ M [methyl-³H] thymidine (20Ci/mmol, New England Nuclear), bombesin (3nM), and initially four concentrations of the test compounds (1, 10, 100, 1000nM) in a final volume of 1.0 ml. After 28 hours at 37°C, [methyl-³H] thymidine incorporation into acid-insoluble pools is assayed as follows. The cells are washed twice with ice-cold 0.9% NaCl (1ml aliquots), and acid soluble radioactivity is removed by a 30 min. (4°C) incubation with 5% trichloroacetic acid

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(TCA). The cultures are then washed once (1ml) with 95% ethanol and solubilized by a 30 min. incubation (1ml) with 0.1N NaOH. The solubilized material is transferred to vials containing 10ml ScintA (Packard), and the radioactivity is determined by liquid scintillation spectrometry.

Phase 2 - Small Cell Carcinoma (SCLC) - Bombesin Stimulated [³H] Thymidine Uptake Assay

Cell Culture. Cultures of the human cell carcinoma cell line (NCI-H69) (obtained from the American Type Culture Association) are maintained in RPMI 1640 medium supplemented with 10% fetal calf serum in 10% CO₂/90% air at 37°C. Twenty-four hours prior to assay, the cells are washed with serum-free medium and seeded in 24-well cluster trays.

Assay of DNA Synthesis. Bombesin (1nM), 0.5μM [methyl-³H] thymidine (20 Ci/mmol, New England Nuclear), and four concentrations of the test compounds (1, 10, 100, 1000nM) are added to the cultures to achieve a final volume of 0.5 ml. After a 28 hr incubation at 37°C, the cells are collected onto GF/B glass fiber filters, and the DNA is precipitated with ice-cold TCA. [³H] thymidine incorporation into acid-insoluble fractions of DNA is determined by liquid scintillation spectrometry.

Phase 3 - Peptide-Induced Pancreatitis

Male, Sprague-Dawley rats (250g) are used for these experiments. The test compound, or 0.9% NaCl is administered s.c. 15 min. prior to the bombesin injection. Bombesin injections are given s.c. at a dose of 10 μg/kg, and blood samples are obtained at 1 hr. 30 min., 3hr. and 6hr. Plasma amylase concentration are determined by the Pantrak Amylase test.

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Phase 4- In Vitro Inhibition of [¹²⁵I] Gastrin
Releasing Peptide (GRP) Binding to Bombesin
Receptors

5 Membranes from various tissues (rat brain, rat
pancreas, rat anterior pituitary, SCLC, 3T3 cells) are
prepared by homogenization in 50mM TrisHCl containing
0.1% bovine serum albumin and 0.1mg/ml bacitracin
followed by two centrifugations (39,000xg x 15 min., 4°C)
with an intermediate resuspension in fresh buffer. For
10 assay, aliquots (0.8ml) are incubated with 0.5nM
[¹²⁵I]GRP ('2000 Ci/mmol, Amersham Corp.) and various
concentrations of the test compounds in a final volume
of 0.5ml. After a 30 minute incubation at 4°C, the
binding reaction is terminated by rapid filtration
15 through Whatman GF/C filters that have been pre-soaked
in 0.3% aqueous polyethyleneimine to reduce the level of
nonspecific binding. The filters and tubes are washed
three times with 4ml aliquots of ice-cold buffer, and
the radioactivity trapped on the filters is counted by
20 gamma-spectrometry. Specific binding is defined as the
total [¹²⁵I]GRP bound minus that bound in the presence
of 1000nM bombesin.

Phase 5- Inhibition of Gastrin Release

25 The stomachs of anesthetized rats are perfused
with saline collected over 15 minute periods via pyloric
cannulation while the test peptide is infused through
the femoral vein for periods between 0 and 150 minutes.

Phase 6- In Vivo Antitumor Activity

30 NCI-H69 small cell lung carcinoma cells were
transplanted from in vitro culture by implanting each
animal with the equivalent of 5 confluent 75 cm²
tissue culture flasks in the right flank. In vitro
NCI-H69 cells grow as a suspension of cellular
aggregates. Therefore, no attempt was made to

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disaggregate the cell agglomerates by physical or chemical means. Tumor size was calculated as the average of two diameters, i.e., (length and width/2) mm.

5 Results of Assays of Test Peptides

 A number of analogs of bombesin or GRP, each containing a non-peptide bond or a statine, AHPPA, or ACHPA residue, can be synthesized and tested in one or more of the above-described Phase 1 - 6 assays; the results of Phase 1 and 2 tests are given in Table 1 attached hereto. Table I shows formulas for the non-peptide analogues and results of in vitro inhibition of [¹²⁵I]GRP binding to 3T3 fibroblast bombesin receptors, and bombesin-stimulated [³H]Thymidine uptake by cultured 3T3 cells. (3T3 GRP receptor and thymidine uptake data are expressed in IC₅₀ (nM).) Table 1 also gives results for non-peptide bond-containing analogs of one other naturally-occurring peptide, Neuromedin C, whose C-terminal seven amino acids are similar to those of bombesin and GRP. (In Table I, "Litorin" indicates a 9 residue peptide analog or its derivative, whereas "Neuromedin C" indicates a 10 residue analog or its derivative.)

 In Table I, the position of the non-peptide bond is indicated by the position of the symbol $\psi[\text{CH}_2\text{NH}]$; i.e., $\psi[\text{CH}_2\text{NH}]$ is always shown preceding the amino acid which, in that peptide, is bonded to the amino acid N-terminal to it via the non-peptide bond. Where no amino acid is specified under "structure", the non-peptide bond links the two peptides represented by the numbers given as post-scripts.

 In Table I, it can be seen that a preferred placement of the non-peptide bond in litorin analogs is

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at the A⁸ - A⁹ position; two of the most active analogs (as indicated by a low GRP receptor IC₅₀ value) are BIM-26100 (Phe⁸ψ[CH₂NH]Leu⁹) and BIM-26101 (Leu⁸ψ[CH₂NH]Leu⁹).

5 In addition, as shown in Table I, BIM-26113 (D-Phe¹, Leu⁸ψ[CH₂NH]Leu⁹) and BIM-26114 (D-Nal¹, Leu⁸ψ[CH₂NH]Leu⁹) are active in the 3T3 GRP receptor binding and thymidine uptake assays. Most notably, BIM-26136 (D-Nal¹,
10 Leu⁸ψ[CH₂NH]Phe⁹), which contains amino and carboxy terminal aromatic residues that are capable of forming a hydrophobic interaction, is the most potent analog. Finally, when statine or β-leucine replaces the A⁸ and A⁹ residues of litorin, the resultant analogs
15 BIM-26120 and BIM-26182 are also potent antagonists.

Table I also shows that Neuromedin C analogs containing a non-peptide bond between residues A⁹ - A¹⁰, e.g., BIM-26092, 26095, 26106, and 26107, are
20 antagonists when tested in the 3T3 GRP receptor and thymidine uptake assays.

Table I also gives negative results for analogs of Neuromedin C, e.g., BIM-26108. Thus the non-peptide bond placement guidelines given herein should be used in conjunction with the routine assays described above to
25 select useful antagonists.

Bombesin and Bombesin analogs have been shown to inhibit the effect of interleukin-2 (IL-2) (Fink et al., 1988, Klin. Wochenschr. 66, Suppl. 13, 273). Since IL-2 causes T lymphocytes to proliferate, it is possible
30 that litorin antagonists may prevent the inhibitory effect of Bombesin or its analogs on IL-2. IL-2 stimulated lymphocytes are capable of effectively lysing small cell lung carcinoma cells in vitro. Although Bombesin antagonists have a direct antiproliferative

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effect on neoplastic tissues, they may also favor proliferation of lymphocytes having lytic activity for small cell lung carcinoma.

These observations prompted us to evaluate the effect of BIM-26100 on the in vivo growth of the SCLC tumor cell line described in Phase 6. Twenty athymic nude females, 5 to 6 weeks of age, were implanted on day 0 with the NCI-H69 human SCLC, individually identified and then randomized into the following vehicle control and test groups:

<u>Group No.</u>	<u>Treatment</u>	<u>No. Animals</u>
1	Saline vehicle treated control: 0.2 ml, s.c. inf., b.i.d., QD1-28	10
2	BIM-26100: 50ug/inj., s.c., b.i.d., QD1-28	5
3	BIM-26100: 50ug/inj., s.c. inf., b.i.d., QD1-28	5

(s.c. = subcutaneously; inj. = injected; b.i.d. = twice per day; QD1-28 = daily treatment, on days 1 - 28.)

Growth of NCI-H69 xenografts and the tumor growth inhibitory activity of the bombesin antagonist BIM-26100 (pGlu-Gln-Trp-Ala-Val-Gly-His-Pheψ[CH₂NH]Leu-NH₂) are illustrated as tumor growth curves in Fig. 1, and relative tumor sizes in Table II. Administration of BIM-26100 as a s.c. infusion around the tumor significantly inhibited tumor growth. The effectiveness of the antitumor activity of BIM-26100 is evident in view of the large inoculum of NCI-H69 tumor cells (i.e., the equivalent of 5 confluent 75 cm² cell culture flasks per animal) and the agglomerated condition of the cells. In confluent flasks, NCI-H69 agglomerates are macroscopically visible and together resemble a metastatic tumor colony. Many such tumor colonies were implanted per animal. The dose of BIM-26100 was

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arbitrarily selected on the basis of compound availability and is not optimal. Higher doses of BIM-26100 may be administered, as indicated by body weight gain (minus tumor weight) gain during the course of treatment (Table III). This suggest BIM-26100 completely lacks local or systemic toxicity and is useful therapeutically as an anti-growth factor with anti-tumor effects.

Fig. 3 shows the effect of the bombesin antagonist
D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH₂NH]Phe-NH₂
on bombesin-stimulated amylase secretion in the rat. The results show that this analog is a potent antagonist; 5 nM of the analog can inhibit the secretion of anylase stimulated by 0.5 nM of bombesin for 150 minutes after bolus injection.

Use

The peptides of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery (e.g., in the case of anti-cancer bombesin to the lungs) using micelles, gels and liposomes.

The bombesin antagonists of the invention are suitable for the treatment of all forms of cancer where bombesin-related substances act as autocrine or paracrine mitotic agents, particularly small-cell lung carcinoma. The peptides can also be used for the inhibition of gastric acid secretion and motility disorders of the GI tract, the symptomatic relief and/or treatment of exocrine pancreatic adenocarcinoma, and the restoration of appetite to cachexic patients. The

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peptides can be administered to a human patient in a dosage of 0.5 µg/kg/day to 5 mg/kg/day. For some forms of cancer, e.g., small cell lung carcinoma, the preferred dosage for curative treatment is

5 250mg/patient/day.

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Table 1

<u>Code</u>	<u>Structure</u>	3T3 GRP Receptor <u>IC50(nM)</u>	Thym. Uptake <u>IC50(nM)</u>
BIM-26092	Gly-Asn-His-Trp-Ala-Val-Gly- His-Leu ψ [CH ₂ NH]Leu-NH ₂ Neuromedin C	242	466
BIM-26095	pGlu-Gln-Trp-Ala-Val-D-Ala- His-Leu ψ [CH ₂ NH]Leu-NH ₂ Litorin	2623	1209
BIM-26100	pGlu-Gln-Trp-Ala-Val-Gly-His- Phe ψ [CH ₂ NH]Leu-NH ₂ Litorin	23	26
BIM-26101	pGlu-Gln-Trp-Ala-Val-Gly-His- Leu ψ [CH ₂ NH]Leu-NH ₂ Litorin	118	296
BIM-26105	D-Ala-Asn-His-Trp-Ala-Val- D-ALA-His-Leu ψ [CH ₂ CH]Leu-NH ₂ Neuromedin C	107	107
BIM-26106	desGly-D-Ala-His-Trp-Ala-Val- D-Ala-His-Leu ψ [CH ₂ NH]Met-NH ₂ Neuromedin C	401	354
BIM-26107	D-Phe-His-Trp-Ala-Val-Gly- His-Leu ψ [CH ₂ NH]Leu-NH ₂ Neuromedin C	199	154

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Table 1 (cont'd)

<u>Code</u>	<u>Structure</u>	3T3 GRP Receptor <u>IC50(nM)</u>	Thym. Uptake <u>IC50(nM)</u>
BIM-26108	N-Ac-D-Ala-His-Trp-Ala-Val-Gly-His-Leu ψ [CH ₂ NH]Leu-NH ₂ Neuromedin C	841	>1000
BIM-26113	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH ₂ NH]Leu-NH ₂ Litorin	5.8	9
BIM-26114	D-Nal-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH ₂ NH]Leu-NH ₂ Litorin	23.5	28
BIM-26120	pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH ₂ Litorin	150	165
BIM-26122	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH ₂ Litorin	5.9	28.6
BIM-26136	D-Nal-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH ₂ NH]Phe-NH ₂ Litorin	1.4	3.3
BIM-26182	D-Cpa-Gln-Trp-Ala-Val-Gly-His- β -Leu-NH ₂ Litorin	0.88	4.77

Table II

**IN VIVO TUMOR INHIBITORY ACTIVITY OF THE BOMBESIN ANTAGONIST BIM-26100:
NCL-H62 HUMAN SCLC**

Group No.	Treatment	Tumor Size ¹ Day 18 (mm)	% Test/Control	Tumor Size Day 28 (mm)	% Test/Control
1	Vehicle treated control, 0.2ml, s.c. inf., b.i.d., QD1-28	10.9±1.82		15.9±2.27	
2	BIM-26100, 50µg/inj., s.c., b.i.d., QD1-28	10.1±1.47	93	17.3±1.96	108
3	BIM-26100, 50µg/inj., s.c. inf., b.i.d., QD1-28	7.6±1.56**	70	13.7±0.67*	86

¹Data reported as means ± SD on 10 animals in the control and 5 in test groups.
Student's t Test significance of difference from control: *p<0.05; **p<0.01.

Table III

**EFFECT OF TUMOR GROWTH AND BIM-26100 TREATMENT ON BODY WEIGHT:
LACK OF SYSTEMIC TOXICITY**

Group No.	Treatment	Body Weight(gm) ¹	
		Day 0	Day 18
1	Vehicle treated control, 0.2ml, s.c. inf., b.i.d., QD1-28	17.3	19.6
2	BIM-26100, 50µg/inj., s.c., b.i.d., QD1-28	16.9	19.2
3	BIM-26100, 50µg/inj., s.c. inf., b.i.d., QD1-28	17.7	20.4
			19.7
			19.1
			21.1

¹ Body weights are presented as means of 10 animals in the control and 5 in test groups. Tumor weights calculated from 2 largest diameters in mm converted to mgs using the formula for an ellipsoid (length x width ²/2)mgs, were subtracted from total body weights.

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Claims

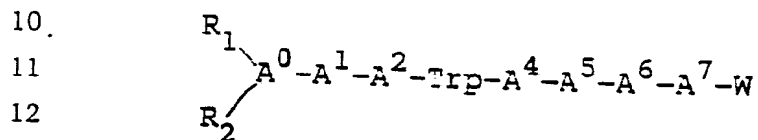
1 1. A linear peptide which is an analog of
2 naturally occurring, biologically active amphibian
3 bombesin or mammalian GRP having an active site and a
4 binding site responsible for the binding of said peptide
5 to a receptor on a target cell, cleavage of a peptide
6 bond in said active site of naturally occurring bombesin
7 or GRP being unnecessary for in vivo biological
8 activity, said analog having one of the following
9 modifications: (a) a deletion of a residue within said
10 active site and a modification of a residue outside of
11 said active site, or (b) a replacement of one or two
12 residues within said active site with a synthetic amino
13 acid, said analog being capable of binding to said
14 receptor so that said analog is capable of acting as a
15 competitive inhibitor of said naturally occurring
16 peptide by binding to said receptor and, by virtue of
17 one of said modifications, failing to exhibit the in
18 vivo biological activity of said naturally occurring
19 bombesin or GRP.

1 2. The linear peptide of claim 1 wherein said
2 active site comprises at least one amino acid in the
3 carboxy terminal half of the peptide, said linear
4 peptide including said amino acid in its carboxy
5 terminal half.

1 3. The linear peptide of claim 1 wherein said
2 active site includes at least one amino acid in the
3 amino terminal half of the peptide, said linear peptide
4 including said amino acid in its amino terminal half.

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1 4. A therapeutic peptide comprising between
 2 seven and nine amino acid residues, inclusive, said
 3 peptide being an analog of one of the following
 4 naturally occurring peptides terminating at the
 5 carboxy-terminus with a Met residue: (a) litorin; (b)
 6 neuromedin; (c) the ten amino acid carboxy-terminal
 7 region of mammalian gastrin releasing peptide; and (d)
 8 the ten amino acid carboxy-terminal region of amphibian
 9 bombesin, said therapeutic peptide being of the formula:



13 wherein

14 $A^0 =$ Gly, Nle, α -aminobutyric acid, or the
 15 D-isomer of any of Ala, Val, Gln, Asn, Leu,
 16 Ile, Met, p-X-Phe (where X = F, Cl, Br, NO₂,
 17 OH, H or CH₃), Trp, Cys, or β -Nal, or is
 18 deleted;

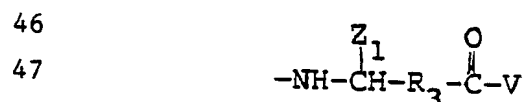
19 $A^1 =$ the D- or L-isomer of any of pGlu, Nle, or
 20 α -aminobutyric acid, or the D-isomer of any
 21 of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe
 22 (where X = F, Cl, Br, NO₂, OH, H or CH₃),
 23 Trp, Cys, or β -Nal, or is deleted;

24 $A^2 =$ pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met,
 25 p-X-Phe (where X = F, Cl, Br, NO₂, OH, H or
 26 CH₃), Trp, Cys, β -Nal, His, 1-methyl-His, or
 27 3-methyl-His;

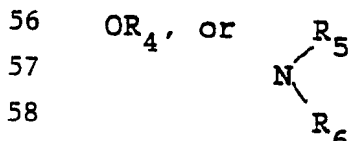
28 $A^4 =$ Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle,
 29 α -aminobutyric acid, Met, p-X-Phe (where X =
 30 F, Cl, Br, NO₂, OH, H or CH₃), Trp, Cys, or
 31 β -Nal;

- 47 -

- 32 $A^5 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 33 α -aminobutyric acid, Met, Val, p-X-Phe (where
 34 X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or
 35 β -Nal;
 36 $A^6 =$ Sar, Gly, or the D-isomer of any of Ala, Val,
 37 Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F,
 38 Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or
 39 β -Nal;
 40 $A^7 =$ 1-methyl-His, 3-methyl-His, or His;
 41 provided that if A^0 is present, A^1 cannot be pGlu;
 42 and if A^0 or A^1 is present, A^2 cannot be pGlu; and
 43 when A^0 is deleted and A^1 is pGlu, R_1 must be H
 44 and R_2 must be the portion of Glu that forms the imine
 45 ring in pGlu; and further provided that W can be:



- 48 wherein R_3 is $CHR_{14}-(CH_2)_{n1}$ (where R_{14} is
 49 either H or OH; and $n1$ may be either of 1 or 0), or is
 50 deleted, and Z_1 is the identifying group of any one of
 51 the amino acids Gly, Ala, cyclohexyl-Ala, Val, Leu, Ile,
 52 Ser, Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F, Cl,
 53 Br, NO_2 , OH, or CH_3), Trp, Cys, Met, Pro, HyPro, or
 54 isopropyl, cyclohexylmethyl, β -nal, β -naphthylmethyl, or
 55 phenylmethyl; and V is either



- 59 where R_4 is any of C_{1-20} alkyl, C_{3-20} alkenyl,
 60 C_{3-20} alkynyl, phenyl, naphthyl, or C_{7-10}
 61 phenylalkyl, and each R_5 , and R_6 , independently, is
 62 any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, lower

- 48 -

63 acyl, or $\begin{array}{c} R_{15} \\ \diagup \\ N \\ \diagdown \\ H \end{array}$

64
65
66 where R_{15} is any of H, C_{1-12} alkyl, C_{7-10}
67 phenylalkyl, or lower acyl; provided that when one of
68 R_5 or R_6 is NHR_{15} , the other is H; and provided
69 that any asymmetric carbon atom can be R, S or a racemic
70 mixture; and further provided that each R_1 and R_2 ,
71 independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl,
72 COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl,
73 C_{3-20} alkynyl, phenyl, naphthyl, or C_{7-10}
74 phenylalkyl), or lower acyl, and R_1 and R_2 are
75 bonded to the N-terminal amino acid of said peptide, and
76 further provided that when one of R_1 or R_2 is
77 COE_1 , the other must be H, or a pharmaceutically
78 acceptable salt thereof.

1 5. The therapeutic peptide of claim 4 wherein
2 A^0 = Gly, D-Phe, or is deleted;
3 A^1 = p-Glu, D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;
4 A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;
5 A^4 = Ala;
6 A^5 = Val;
7 A^6 = Sar, Gly, D-Phe, or D-Ala;
8 A^7 = His;
9 provided that where R_3 is CH_2-CH_2 , Z_1 is the
10 identifying group of Leu or Phe; or where R_3 is CH_2 ,
11 Z_1 is the identifying group of β-Leu or Leu; or where
12 R_3 is $CHOH-CH_2$, Z_1 is the identifying group of Leu
13 or is isopropyl, cyclohexylmethyl, β-naphthylmethyl, or
14 phenylmethyl; provided that where V is $\begin{array}{c} R_5 \\ \diagup \\ N \\ \diagdown \\ R_6 \end{array}$
15
16
17 each R_5 and R_6 is H.

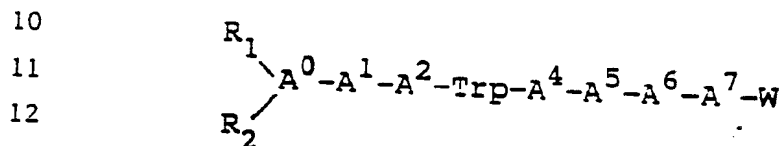
- 49 -

1 6. The therapeutic peptide of claim 5 wherein
2 V is NHR_6 and R_6 is H.

1 7. The therapeutic peptide of claim 5 of the
2 formula:
3 pGlu-Gln-Trp-Ala-Val-Gly-His-statine-amide.

1 8. The therapeutic peptide of claim 5 of the
2 formula:
3 D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His- β -Leu- NH_2 .

1 9. A therapeutic peptide comprising between
2 eight and ten amino acid residues, inclusive, said peptide
3 being an analog of one of the following naturally
4 occurring peptides terminating at the carboxy-terminus
5 with a Met residue: (a) litorin; (b) neuromedin; (c) the
6 ten amino acid carboxy-terminal region of mammalian
7 gastrin releasing peptide; and (d) the ten amino acid
8 carboxy-terminal region of amphibian bombesin, said
9 therapeutic peptide being of the formula:



13 wherein
14 A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer
15 of any of Ala, Val, Gln, Asn, Leu, Ile, Met,
16 p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or
17 CH_3), Trp, Cys, or β -Nal, or is deleted;

- 50 -

18 $A^1 =$ the D- or L-isomer of any of pGlu, Nle, or
 19 α -aminobutyric acid, or the D-isomer of any of
 20 Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where
 21 $X = F, Cl, Br, NO_2, OH, H$ or CH_3), Trp, Cys,
 22 or β -Nal, or is deleted;

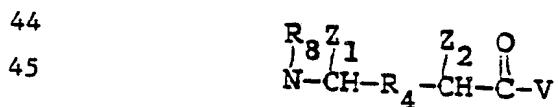
23 $A^2 =$ pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met,
 24 p-X-Phe (where $X = F, Cl, Br, NO_2, OH, H$ or
 25 CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or
 26 3-methyl-His;

27 $A^4 =$ Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle,
 28 α -aminobutyric acid, Met, p-X-Phe (where $X = F,$
 29 Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or
 30 β -Nal;

31 $A^5 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 32 α -aminobutyric acid, Met, Val, p-X-Phe (where X
 33 $= F, Cl, Br, OH, H$ or CH_3), Trp, Thr, or β -Nal;

34 $A^6 =$ Sar, Gly, or the D-isomer of any of Ala, Val,
 35 Gln, Asn, Leu, Ile, Met, p-X-Phe (where $X = F,$
 36 Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or
 37 β -Nal;

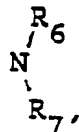
38 $A^7 =$ 1-methyl-His, 3-methyl-His, or His;
 39 provided that if A^0 is present, A^1 cannot be pGlu; and
 40 if A^0 or A^1 is present, A^2 cannot be pGlu; and when
 41 A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2
 42 must be the portion of Glu that forms the imine ring in
 43 pGlu; and further provided that W can be:



46 wherein R_4 is CH_2-NH , CH_2-S , $CO-CH_2$, or
 47 CH_2-CH_2 , and each of Z_1 and Z_2 , independently, is
 48 the identifying group of any one of the amino acids Gly,
 49 Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, β -Nal,

- 51 -

50 p-X-Phe (where X = H, F, Cl, Br, NO₂, OH or CH₃), Trp,
 51 Cys, Met, Pro, HyPro, cyclohexyl-Ala, or cyclohexylmethyl;
 52 provided that where R₄ is CH₂-NH and Z₂ is the
 53 identifying group of any one of the amino acids Gly, Ala,
 54 Val, Leu,
 55 Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F,
 56 Cl, Br, NO₂, OH or CH₃), Trp, Cys, Met, Pro, HyPro,
 57 or cyclohexylmethyl, Z₁ can only be the identifying
 58 group of any one of the amino acids Ser, Asp, Glu, Cys,
 59 Pro, HyPro, or cyclohexylmethyl; and provided that where
 60 R₄ is CH₂-NH and Z₁ is the identifying group of
 61 any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser,
 62 Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F, Cl, Br,
 63 NO₂, OH or CH₃), Trp, Cys, Met, Pro, or HyPro, Z₂
 64 can only be the identifying group of any one of the
 65 amino acids Ser, Asp, Glu, Cys, Pro, HyPro, or
 66 cyclohexylmethyl; and V is either OR₅ or



70 where each R₈, R₅, R₆, and R₇, independently, is
 71 H, lower alkyl, lower phenylalkyl, or lower
 72 naphthylalkyl; and provided that any asymmetric carbon
 73 atom can be R, S or a racemic mixture; and further
 74 provided that each R₁ and R₂, independently, is H,
 75 C₁₋₁₂ alkyl, C₇₋₁₀ phenylalkyl, COE₁ (where E₁
 76 is C₁₋₂₀ alkyl, C₃₋₂₀ alkenyl, C₃₋₂₀ alkynyl,
 77 phenyl, naphthyl, or C₇₋₁₀ phenylalkyl), or lower
 78 acyl, and R₁ and R₂ are bonded to the N-terminal
 79 amino acid of said peptide, and further provided that
 80 when one of R₁ or R₂ is COE₁, the other must be H,
 81 or a pharmaceutically acceptable salt thereof.

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1 10. The therapeutic peptide of claim 9 wherein
2 A^0 = Gly, D-Phe, or is deleted;
3 A^1 = p-Glu, D-Phe, D-Ala, D- β -Nal, D-Cpa, or D-Asn;
4 A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;
5 A^4 = Ala;
6 A^5 = Val;
7 A^6 = Sar, Gly, D-Phe, or D-Ala;
8 A^7 = His;
9 where R_4 is CH_2-NH , each Z_1 is cyclohexylmethyl or
10 is the identifying group of Leu or Phe; or Z_2 is the
11 identifying group of Met, Leu or Phe.

1 11. The therapeutic peptide of claim 9 wherein
2 A^1 is D- β -Nal, each of Z_1 and Z_2 , independently,
3 is Leu or Phe.

1 12. The therapeutic peptide of claim 11 of the
2 formula:
3 D- β -Nal-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH_2NH]Leu-NH₂.

1 13. The therapeutic peptide of claim 11 of the
2 formula:
3 D- β -Nal-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH_2NH]Phe-NH₂.

1 14. The therapeutic peptide of claim 9 wherein
2 R_4 is CH_2-NH , and said carbon atom bonded to Z_2 is
3 of said R configuration.

1 15. The therapeutic peptide of claim 14 of the
2 formula
3 D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu ψ [CH_2NH]-D-Phe-NH₂.

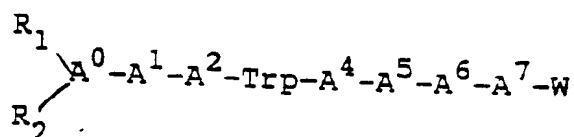
1 16. The therapeutic peptide of claim 9 where
2 A^0 or A^1 is a D amino acid and V is OR_4 .

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17. The therapeutic peptide of claim 16 of the formula:

D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Met-methylester.

18. A therapeutic peptide comprising between seven and nine amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) neuromedin; (c) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (d) the ten amino acid carboxy-terminal region of amphibian bombesin, said therapeutic peptide being of the formula:



wherein

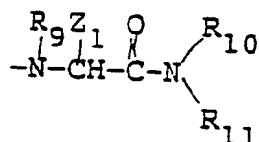
A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

A^1 = the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;

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- 28 $A^4 =$ Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle,
 29 α -aminobutyric acid, Met, p-X-Phe (where X =
 30 F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or
 31 β -Nal;
 32 $A^5 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 33 α -aminobutyric acid, Met, Val, p-X-Phe (where
 34 X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or
 35 β -Nal;
 36 $A^6 =$ Sar, Gly, or the D-isomer of any of Ala, Val,
 37 Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F,
 38 Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or
 39 β -Nal;
 40 $A^7 =$ 1-methyl-His, 3-methyl-His, or His;
 41 provided that if A^0 is present, A^1 cannot be pGlu;
 42 and if A^0 or A^1 is present, A^2 cannot be pGlu; and
 43 when A^0 is deleted and A^1 is pGlu, R_1 must be H
 44 and R_2 must be the portion of Glu that forms the imine
 45 ring in pGlu; and further provided that W can be:



- 49 wherein Z_1 is the identifying group of any one of the
 50 amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu,
 51 Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2 , OH or
 52 CH_3), Trp, Cys, Met, Pro, or HyPro; and each R_9 ,
 53 R_{10} , and R_{11} , independently, is H, lower alkyl,
 54 lower phenylalkyl, or lower naphthylalkyl; and provided
 55 that any asymmetric carbon atom can be R, S or a racemic
 56 mixture; and further provided that each R_1 and R_2 ,
 57 independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl,
 58 COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl,
 59 C_{3-20} alkynyl, phenyl, naphthyl, or C_{7-10}

- 55 -

60 phenylalkyl), or lower acyl, and R_1 and R_2 are
61 bonded to the N-terminal amino acid of said peptide, and
62 further provided that when one of R_1 or R_2 is
63 COE_1 , the other must be H, or a pharmaceutically
64 acceptable salt thereof.

1 19. The therapeutic peptide of claim 18 wherein
2 A^0 = Gly, D-Phe, or is deleted;
3 A^1 = p-Glu, D-Phe, D-Ala, D- β -Nal, D-Cpa, or D-Asn;
4 A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;
5 A^4 = Ala;
6 A^5 = Val;
7 A^6 = Sar, Gly, D-Phe, or D-Ala;
8 A^7 = His;
9 provided that Z_1 is the identifying group of any one
10 of the amino acids Leu or D or L p-X-Phe (where X = H,
11 F, Cl, Br, NO_2 , OH or CH_3); and each R_9 , R_{10} and
12 R_{11} , independently, is H, lower alkyl, lower
13 phenylalkyl, or lower naphthylalkyl.

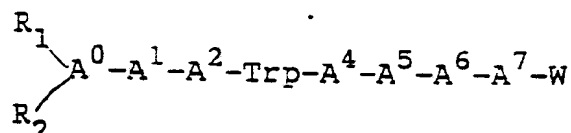
1 20. The therapeutic peptide of claim 19
2 wherein Z_1 is Leu, R_9 is H, and each R_{10} and R_{11}
3 is lower alkyl.

1 21. The therapeutic peptide of claim 20 of the
2 formula:
3 D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ethylamide.

1 22. The therapeutic peptide of claim 20 of the
2 formula:
3 D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu- NH_2 .

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23. A therapeutic peptide comprising between six and eight amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) neuromedin; (c) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (d) the ten amino acid carboxy-terminal region of amphibian bombesin, said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

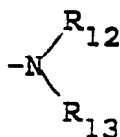
A^1 = the D- or L-isomer of any of pGlu, Nle, or α -aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, β -Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α -aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or β -Nal;

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32 $A^5 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 33 α -aminobutyric acid, Met, Val, p-X-Phe (where
 34 X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or
 35 β -Nal;
 36 $A^6 =$ Sar, Gly, or the D-isomer of any of Ala, Val,
 37 Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F,
 38 Cl, Br, NO_2 , OH, H or CH_3), Trp, Cys, or
 39 β -Nal;
 40 $A^7 =$ 1-methyl-His, 3-methyl-His, or His;
 41 provided that if A^0 is present, A^1 cannot be pGlu;
 42 and if A^0 or A^1 is present, A^2 cannot be pGlu; and
 43 when A^0 is deleted and A^1 is pGlu, R_1 must be H
 44 and R_2 must be the portion of Glu that forms the imine
 45 ring in pGlu; and further provided that W can be:



49 wherein each R_{12} and R_{13} , independently, is H, lower
 50 alkyl, lower phenylalkyl, or lower naphthylalkyl;
 51 provided that any asymmetric carbon atom can be R, S or
 52 a racemic mixture; and further provided that each R_1
 53 and R_2 , independently, is H, C_{1-12} alkyl, C_{7-10}
 54 phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl,
 55 C_{3-20} alkenyl, C_{3-20} alkynyl, phenyl, naphthyl, or
 56 C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2
 57 are bonded to the N-terminal amino acid of said peptide,
 58 and further provided that when one of R_1 or R_2 is
 59 COE_1 , the other must be H, or a pharmaceutically
 60 acceptable salt thereof.

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1 24. The therapeutic peptide of claim 23 wherein

2 A^0 = Gly, D-Phe, or is deleted;

3 A^1 = p-Glu, D-Phe, D-Ala, D- β -Nal, D-Cpa, or D-Asn;

4 A^2 = Gln, His, 1-methyl-His, or 3-methyl-His;

5 A^4 = Ala;

6 A^5 = Val;

7 A^6 = Sar, Gly, D-Phe, or D-Ala;

8 A^7 = His;

9 where each R_{12} and R_{13} , is H; and each R_1 and

10 R_2 , independently, is H, lower alkyl, or lower acyl.

1 25. The therapeutic peptide of claim 24

2 wherein either of N_{12} or N_{13} is other than H, A^7

3 must be His, A^6 must be Gly, A^5 must be Val, A^4

4 must be Ala, and A^2 must be His.

1 26. The therapeutic peptide of claim 24

2 wherein either of R_1 or R_2 is other than H, A^1

3 must not be deleted.

1 27. The therapeutic peptide of claim 4, 9, 18,

2 or 23 wherein said analog is at least 25% homologous

3 with said naturally occurring peptide.

1 28. The therapeutic peptide of claim 27

2 wherein said analog is at least 50% homologous with said

3 naturally occurring peptide.

1 29. A bombesin therapeutic peptide of the

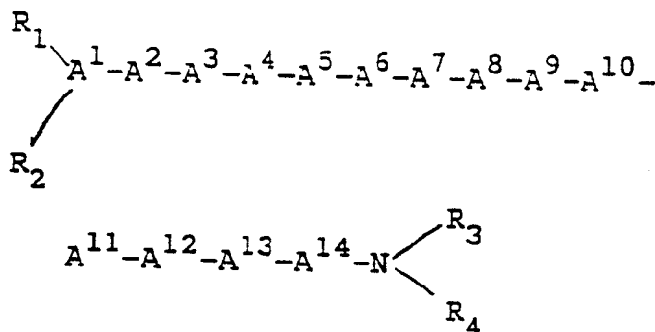
2 formula:

3 pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-

4 Statine.

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30. An effective bombesin antagonistic peptide containing the amino acid formula:



wherein

- A^1 = pGlu, D or L, or is deleted;
 A^2 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, Phe, p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp, β -naphthylalanine or is deleted;
 A^3 = Arg, D-Arg, Lys, D-Lys or is deleted;
 A^4 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, Phe, p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp, β -naphthylalanine or is deleted;
 A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, Phe, D-Phe, p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp, β -naphthylalanine, D-Ala or is deleted;
 A^6 = Gln, Asn, Gly, Ala, D-Ala, N-Ac-D-Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, Phe, p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp, p-Glu, β -naphthylalanine or is deleted;
 A^7 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α -aminobutyric acid, Met, Val, Phe, D-Phe, p-X-Phe (X = F, Cl, Br, OH or CH₃), Trp, Lys, His, or β -naphthylalanine;
 A^8 = Trp or Met;

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33 $A^9 =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 34 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 35 (X = F, Cl, Br, OH or CH_3), Trp, or
 36 β -naphthylalanine, D or L;
 37 $A^{10} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 38 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 39 (X = F, Cl, Br, OH or CH_3), Trp, Thr, or
 40 β -naphthylalanine;
 41 $A^{11} =$ Gly, Phe, D or L;
 42 $A^{12} =$ His, Phe, or p-X-Phe (X = F, Cl, Br, OH,
 43 CH_3), D or L;
 44 $A^{13} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 45 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 46 (X = F, Cl, Br, OH or CH_3), Trp, or
 47 β -naphthylalanine;
 48 $A^{14} =$ Gln, Asn, Gly, Ala, Leu, Ile, Nle,
 49 α -aminobutyric acid, Met, Val, Phe, p-X-Phe
 50 (X = F, Cl, Br, OH or CH_3), Trp, or
 51 β -naphthylalanine;
 52 provided that
 53 each R_1 , R_2 , R_3 , and R_4 , independently,
 54 is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where
 55 E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkynyl,
 56 phenyl, naphthyl, or C_{7-10} phenylalkyl), or $COOE_2$
 57 (where E_2 is C_{1-10} alkyl or C_{7-10} phenylalkyl),
 58 and R_1 and R_2 are bonded to the N-terminal amino
 59 acid of said peptide, which can be A^1 , A^2 , A^3 ,
 60 A^4 , A^5 , A^6 , or A^7 , and further provided that
 61 when one of R_1 or R_2 is COE_1 or $COOE_2$, the other
 62 must be H, and when one of R_3 or R_4 is COE_1 or
 63 $COOE_2$, the other must be H, and further provided that
 64 when $A^1 = pGlu$, R_1 must be H and R_2 must be the
 65 portion of Glu that forms the imine ring in pGlu; and
 66 for each of the residues A^7 , A^8 , A^9 , A^{11} , A^{12} ,

- 61 -

67 and A¹³, independently, the carbon atom participating
68 in the amide bond between that residue and the nitrogen
69 atom of the alpha amino group of the adjacent amino acid
70 residue may be a carbonyl carbon or may be reduced to a
71 methylene carbon, provided that at least one such carbon
72 atom must be reduced to a methylene carbon,

73 said peptide further comprising ()

74 A⁵ = Cys;

75 A⁶ = Cys or a D-isomer of any of said amino acids;

76 A⁷ = pGlu, Cys, 1-methyl-His, or 3-methyl-His;

77 A⁹ = Cys;

78 A¹¹ = Sar, or the D-isomer of any of Ala, Val, Gln,
79 Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO₂,
80 OH, or CH₃), Trp, Cys, or β-Nal;

81 A¹² = 1-methyl-His, or 3-methyl-His;

82 and where A¹⁴ may be deleted.

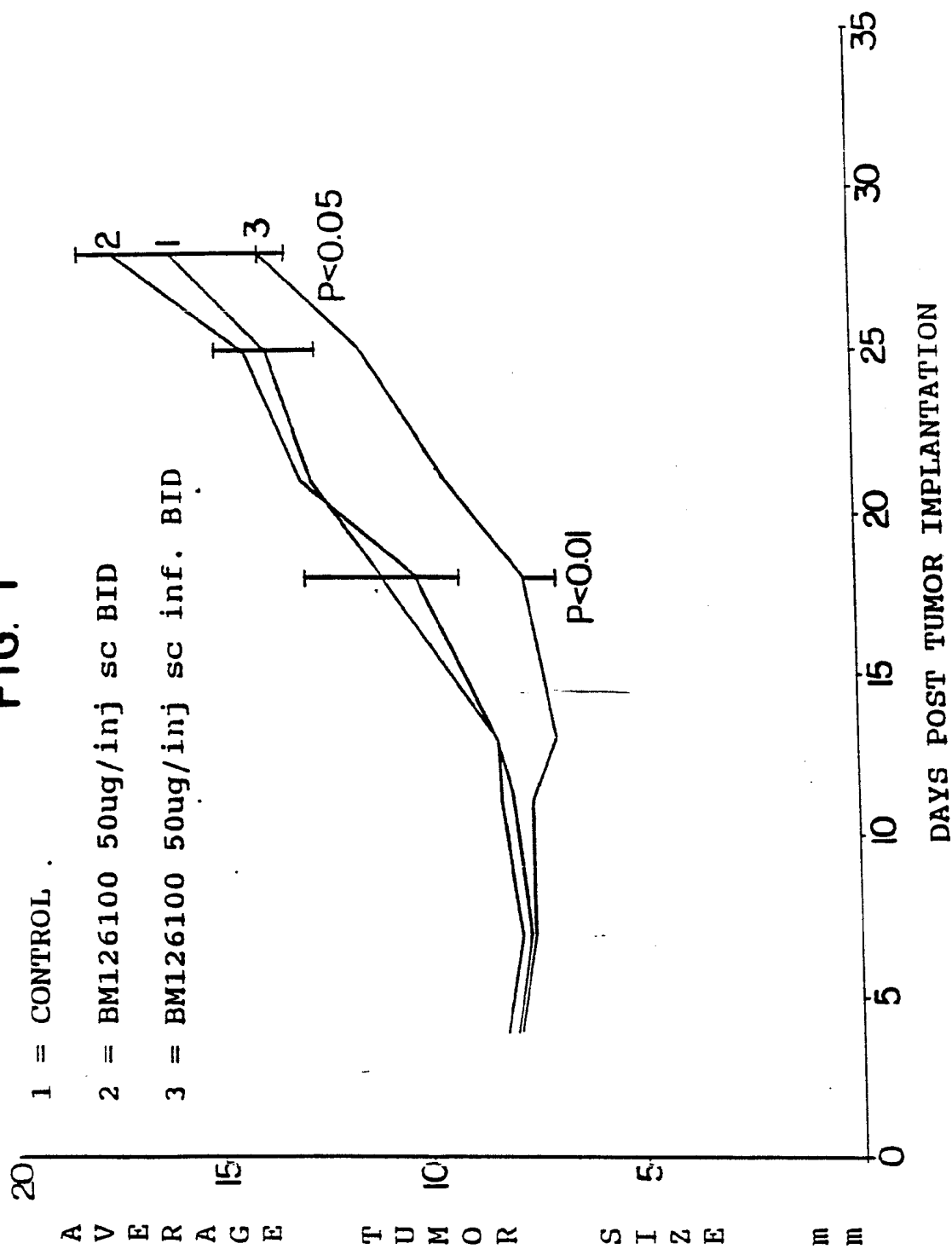
83

84 31. The therapeutic peptide of claim 30 of the
85 formula:

86 D-p-Cl-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH₂NH]Phe-NH₂

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FIG. 1



SUBSTITUTE SHEET

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Litorin

A1 A2 A3 A4 A5 A6 A7 A8 A9
pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met
W

Neuromedin C

A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Ser-His-Trp-Ala-Val-Gly-His-Leu-Met
W

Bombesin (last 10 amino acids)

A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met
W

human GRP (last 10 amino acids)

A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met
W

FIG. 2

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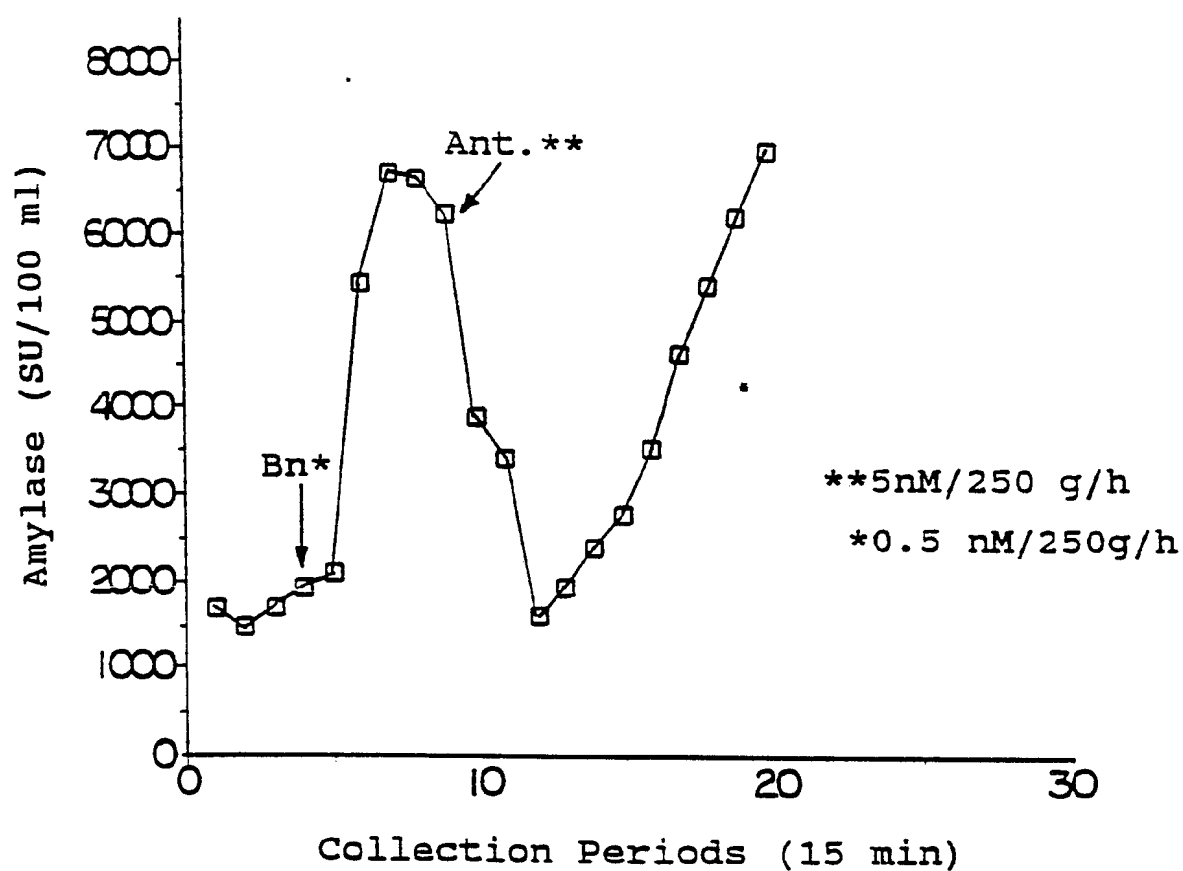


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04616

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶	
According to International Patent Classification (IPC) or to both National Classification and IPC	
IPC(4): C07K 7/02, 7/06, 7/08, 7/10, 7/30	
U.S.Cl.: 530/300, 309, 323, 324, 325, 326, 327, 328, 329	
II. FIELDS SEARCHED	
Minimum Documentation Searched ⁷	
Classification System ¹	Classification Symbols
U.S.	530/300, 309, 323, 324, 325, 326, 327, 328, 329
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸	

CHEMICAL ABSTRACTS AND BIOLOGICAL ABSTRACTS ONLINE COMPUTER SEARCH.

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US, A, 4,207,311 (Brown et al.), 10, June 1980. See column 2, line 29 and claim 1 in particular.	9
X	US, A, 4,331,661 (Marki et al.), 25, May 1982. See the abstract in par- ticular.	9
X	US, A, 4,613,586 (Barchas et al.), 23, September 1986. See table 1, lines 8-10 in particular.	9
P, A	US, A, 4,803,261 (Coy et al.), 07, February 1989.	1-31
X	Am J. of Physiol. (Maryland, USA), issued 1986, (Heinz-Erian et al.), "[D-phe ¹²] bom- besin analogues: a new class of bombesin receptor antagonists", pages G 439-G442. See the abstract in particular.	1-3

⁹ Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not
considered to be of particular relevance

"E" earlier document but published on or after the international
filing date

"L" document which may throw doubts on priority claim(s) or
which is cited to establish the publication date of another
citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or
other means

"P" document published prior to the international filing date but
later than the priority date claimed

"T" later document published after the international filing date
or priority date and not in conflict with the application but
cited to understand the principle or theory underlying the
invention

"X" document of particular relevance: the claimed invention
cannot be considered novel or cannot be considered to
involve an inventive step

"Y" document of particular relevance: the claimed invention
cannot be considered to involve an inventive step when the
document is combined with one or more other such docu-
ments, such combination being obvious to a person skilled
in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of this International Search Report

06 December 1989

11 JAN 1990

International Searching Authority

Signature of Authorized Officer

TSA/UIS

Christina Chan
Christina Chan

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X Proc. Natl. Acad. Sci. USA (Washington, D.C., USA) volume 82, issued November, 1985. (Zachary et al.), "High-affinity receptors for peptides of the bombesin family in Swiss 3T3 cells", pages 7616-7620. See Table 2, Neuromedin C and B in particular.

9

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____ because they relate to subject matter^{1,2} not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out^{1,2}, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING³

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority : : : :
invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X Y	Cancer Surveys (Oxford, England) volume 4, no. 4, issued 1985 (Cuttitta et al.), "Autocrine growth factors in human small cell lung cancer", pages 707-727. See page 718 and Table 2 in particular.	1-3,9, 10,30-31
Y	J. Med. Chem. (Washington, D.C., USA) volume 30 issued 1987 (Sasaki et al.) "Solid-phase synthesis and biological Properties of Ψ [CH ₂ NH] Pseudopeptide analogues of a highly Potent somatostatin octapeptide", pages 1162-1166. See pages 1162, 1164, 1166 in particular.	10,30-31
X	Chemical Abstract, (Columbus, Ohio, USA) volume 109, issued 1988, (Coy et al.) "Probing peptide backbone function in bombesin. A reduced peptide bond analog with potent and specific receptor antagonist activity", the abstract No. 32216K, J. Biol. Chem. 1988, 263 (11), 5056-60 (Eng).	1-3,30
X	Chemical Abstract, (Columbus, Ohio, USA) volume 109, issued 1988, (Woll et al.), "[Leu ¹³ - Ψ (CH ₂ NH) Leu ¹⁴] bombesin is a special bombesin receptor antagonist in Swiss 3T3 cells", the abstract no 163928s, Biochem. Biophys. Res. Commun. 1988, 155(1), 359-65(Eng).	1-3,30